INFECTION AND IMMUNITY, May 1976, p. 1517-1518

Vol. 13, No. 5
Printed in U.S.A.

NOTES

Salmonellosis in Mice: Immunization Experiments with *Salmonella-Escherichia coli* Hybrids

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Received for publication 25 November 1975

Mice vaccinated with *Escherichia coli* hybrids expressing *Salmonella* O antigens were protected against lethal challenge doses of *Salmonella* strains. Counts of challenge bacteria in mouse organs revealed that a live hybrid vaccine was slightly more efficient than an acetone-killed vaccine.

The present investigation is part of an approach to developing safe, live *Salmonella* vaccines. In this study we examined the protective capacity against mouse typhoid of *Escherichia coli* hybrids that received the *Salmonella* O antigens factors 4,5,12 or 9,12 by genetic transfer from *S. typhimurium* and *S. typhi* donors, respectively, as described previously (5). The immunizing effect of living *E. coli* hybrids given via the oral route will be discussed elsewhere; we describe here experiments with mice immunized and challenged by the intraperitoneal (i.p.) route.

For immunization of mice (NMRI) we used the *E. coli* hybrids F1097 and F1193 and the *E. coli* O8 parental strain, which are characterized in Table 1. The ability to ferment lactose and to produce indole are typical traits of *E. coli*. As challenge strain for mice vaccinated with F1097 (O4,5,12) we used *S. typhimurium* SF1826 (mean lethal dose $\sim 10^2$). The protective capacity of the hybrid F1193 (O9,12) that received the O-antigenic outfit from *S. typhi* could not be determined by challenge with *S. typhi*, since this strain is avirulent for mice. However, *S. typhimurium* hybrids with *Salmonella typhi* O9,12 antigens are as virulent as *S. typhimurium* parent strains and most useful in testing the efficiency of typhoid vaccines in mice (3). Such a *S. typhimurium* hybrid (SF10187) was used as challenge for mice immunized with the hybrid F1193.

Groups of 10 to 20 mice were immunized i.p. with about 10^7 living cells of the *E. coli* hybrids F1097 and F1193 and the parental *E. coli* O8 strain. Four weeks after immunization the vaccinated mice and untreated mice (as control) were infected i.p. with 10^5 or 10^6 cells of pathogenic challenge strains. Survivors were recorded for 30 days postchallenge. The results are presented in Table 2. Control mice as well as mice vaccinated with *E. coli* O8 died within a period from days 3 to 8 when challenged with 10^5 cells and within 2 to 6 days after infection with 10^6 cells of the pathogenic challenge strains. In contrast, mice immunized with living *E. coli* hybrids were significantly protected (Table 2).

From these results we can assume that the *Salmonella* O antigens on the cell surface account for the capacity of the *E. coli* hybrids to protect mice against lethal *Salmonella* infections.

It is generally accepted that live *Salmonella* cells, due to their ability to stimulate the cellular defense mechanisms, are more efficient vaccines than killed cells (1, 2, 4). Although the application of killed vaccines results in the survival of a high proportion of mice, it does not inhibit—in contrast to live vaccine—progressive multiplication of challenge bacteria (1). It was of interest to examine whether differences in this respect also exist between live and killed vaccines prepared from *E. coli*

TABLE 1. *Relevant properties of E. coli hybrids and the parental E. coli O8*

Strain	Lactose utilization	Indole formation	O antigens	Mean lethal dose
Salmonella				
F1097	+	+	4,5,12	$>10^7$
F1193	+	+	9,12	$>10^7$
E. coli				
O8	+	+	8	$>10^7$

TABLE 2. *Protection test in mice*

Immunization i.p. with 10^7 living cells of	Survivors/total after i.p. challenge with:			
	S. typhimurium (O4,5,12)		*S. typhimurium* hybrid (O9,12)	
	10^5 [a]	10^6	10^5	10^6
F1097 (04,5,12)	20/20	20/20		
F1193 (09,12)			20/20	12/15
E. coli O8	0/10		0/10	
Control	0/20	0/20	0/20	0/15

[a] Number of cells/dose.

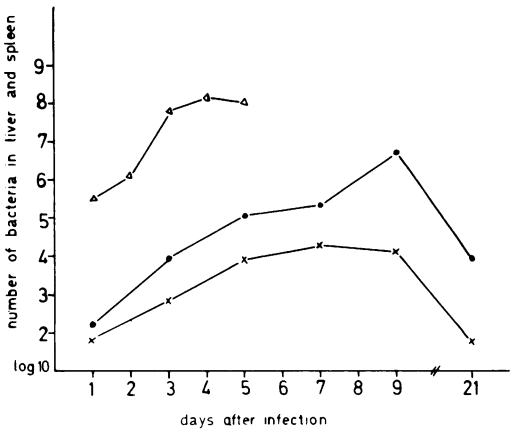

FIG. 1. *Growth curves of S. typhimurium SF1826 in liver and spleen of mice preimmunized i.p. with 10^7 live cells (×) or 10^8 acetone-killed cells (●) of hybrid F1097 and of normal untreated mice (△). Infection dose, 8 × 10^4 cells i.p.*

hybrids. Therefore, one group of mice was vaccinated i.p. with about 10^7 living cells, and for comparison a second group was vaccinated with about 10^8 acetone-killed cells of the *E. coli* hybrid F1097 (O4,5,12). Four weeks later, the vaccinated and nonvaccinated mice (as control) were challenged i.p. with 10^5 cells of *S. typhimurium* SF1826. At intervals (indicated in Fig. 1) five mice from each group were killed, and the number of challenge bacteria in the homogenized livers and spleens was determined. The results are presented in Fig. 1. In control mice the number of challenge bacteria rapidly increased up to 10^8 by day 3. Six days after challenge all of the control mice had died. After a primary reduction the number of challenge bacteria multiplied progressively up to nearly 10^7, by day 9, in mice vaccinated with killed hybrids. From that point on to day 12 some of the mice died, indicating that the challenge bacteria had extensively multiplied. The number of challenge bacteria in mice vaccinated with living hybrids increased from 10^2 up to above 10^4, but never reached the critical population size leading to death of animals.

Experiments are in progress to find out whether the slightly better efficiency of live *E. coli* hybrids is due to stimulation of cellular defense components.

This research was supported by a grant from the Deutsche Forschungsgemeinschaft.

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